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ATTRACTANT OR PHEROMONE: THE CASE OF NASONOV SECRETION AND HONEYBEE SWARMS

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Abstract—Honeybees are attracted to a variety of odors, including the secretion of their Nasonov glands, a secretion that has been widely assumed to be an orientation and attraction pheromone. A crossover design experiment comparing synthetic Nasonov secretion with linalool, oil of clove, skatole, and wax moth sex pheromone was established to determine if Nasonov secretion serves as a true pheromone or is simply a general attractant for honeybee swarms. None of the test odors was more attractive than odorless controls, and in all comparisons, synthetic Nasonov secretion was significantly more attractive than the test odors or odorless controls. The results confirm that Nasonov secretion is a true pheromone in the context of attracting honeybee swarms to nest cavities and that environmentally present or apparent odors play little or no role in honeybee nest-seeking behavior.

Key Words—*Apis mellifera*, linalool, skatole, clove, undecanal, nonanal, citral, geraniol, nerolic acid, geranic acid.

INTRODUCTION

A pheromone is a chemical signal released by an individual of a species for the purpose of eliciting in one or more other individuals of that species a response that is adaptive to both the releasing and receiving individuals. An attractant is simply a chemical that attracts an individual to it. An attractant need not benefit the individual attracted: indeed, it might benefit, harm, or be neutral to the attracted individual. A pheromone can be an attractant, but an attractant often is not a pheromone. Unless an attractant is emitted by a conspecific and designed to elicit a specific response from the receiver, it is not a pheromone—it might be a kairomone, a pheromone mimic, or some other odor present in the

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environment – but it is not a pheromone. To demonstrate that a chemical signal is truly a pheromone, we must be able to demonstrate that it is produced by one individual, is detected by a conspecific, and elicits a specific response in the receiving individual. The burden of proof that a chemical signal is a pheromone is rigorous and often difficult. In the case of attractants that are odors produced by the species of concern, first impressions might suggest that the odor is a pheromone, a conclusion that is not necessarily valid.

Nasonov secretion, a blend of six monoterpenes plus (*E*,*E*)-farnesol (Pickett et al., 1980) is widely regarded as the master honeybee attractant pheromone (Free, 1987). Its various postulated pheromonal roles include: attracting bees to form a cluster during the swarming process; marking the entrance of a new nest cavity or a new entrance to the existing hive, leading nestmates from the parent colony to the new nest cavity during swarming; marking and attracting nestmates to a queen that landed during the swarm flight; and scent-marking sources of water or sugar syrup that possess little odor. In general, Nasaonov secretion is considered to function to orient disoriented bees. We have used synthetic Nasonov pheromone as the key element in swarm traps that are highly attractive to honeybee swarms (Schmidt and Thoenes, 1987) and demonstrated that identical traps without pheromone attracted only 21% as many swarms as traps containing pheromone (Schmidt, 1994). These results suggested that Nasonov "pheromone" was, indeed, a pheromone that attracted other bees to potential nest sites. However, Wells et al. (1993) tested the assumption that Nasonov was a pheromone that attracted foragers to water or sugar sources. Of five tested odors, they discovered that cinnamon oil and cajeput oil when added to sugar syrup induced a larger increase in forager recruitment than the three Nasonov components, citral, geraniol, and nerol. After training to clove oil-scented syrup followed by syrup removal, foragers chose syrups in the order of oil of bay, anise odor, cajeput, clove oil, and finally Nasonov mixture (consisting of 1 : 1 : 0.5 citral, geraniol, and nerol). In a final test, bees were trained to syrup containing a blend of anise, bay, citral, geraniol, and nerol. On subsequent days when given a choice of the syrups containing individual scents, the order of choice by foragers was bay, geraniol, anise, and nerol, with citral being rejected. These data led Wells et al. (1993) to conclude that neither Nasonov secretion nor the components of the Nasonov secretion were a pheromone to attract bees to water or syrup. The authors suggested that in the situation of foragers at liquids, Nasonov secretion simply acted as an added odor to enable other foragers to find the source: it was not better than other odors; it simply was available as a means to impart an odor at the liquid source.

The work of Wells et al. (1993) raises doubts about the assumption that Nasonov secretion is a pheromone that governs nest-seeking and swarming behavior in honeybees. The goal of the research reported here was to test the null and alternative hypotheses: H_0 = Nasonov "pheromone" is simply an odor that enables

scout bees to detect potential nest cavities; H_a = Nasonov pheromone acts as a true attracting and orienting pheromone during the nest-seeking behavior.

METHODS AND MATERIALS

Swarm traps constructed of wood pulp were established in 20 locations within the Tucson, Arizona basin during 1997 and 1998. The traps had an internal volume of 31 liters and were in the shape of inverted truncated cones measuring 40 cm top diameter, 25 cm bottom diameter, and 40 cm high, each with a 3-cm-diameter hole at the bottom (Schmidt et al., 1989). Test odor lures consisted of: Nasonov mixture [1 : 1 : 1 (*E*) and (*Z*)-citral, geraniol, nerolic + geranic acids], linalool, skatole (3-methylindole), clove oil, and wax moth (*Galleria mellonella*) sex pheromone (3 : 1 nonanal: undecanal) (Dickens et al., 1986). The Nasonov mixture was selected as the maximally attractive Nasonov blend based on the work of Free et al. (1982). Those workers showed that the five terpenes blended in the 1 : 1 : 1 ratio were superior in attracting honeybees to cluster than all seven natural pheromone components [the five above, plus nerol and (*E*,*E*)-farnesol] combined in either equal proportions or in their natural ratios. Geraniol was obtained from Aldrich Chem. Co., Milwaukee, Wisconsin, nerolic + geranic acids from Bedoukian Research, Danbury, Connecticut, and the rest of the chemicals were from Sigma Chemical Co., St. Louis, Missouri. Each lure consisted of two 250-μl sealed polyethylene microtubes (Bio-Rad, Richmond, California) containing 100 μl each of the test material. The lures were then wrapped in black porous paper and attached with tacks inside the swarm traps just above the entrance hole. The rationale for the test odors was: Nasonov mixture is the putative pheromone; linalool is a highly attractive floral odor for bees; skatole likely would be present from the feces in the bottom of tree cavities inhabited by animals and it might be a deterrent odor; clove oil is a strong nonfloral plant odor; and wax moth sex pheromone provides an indication that a previous honeybee colony had sucessfully inhabited the cavity. These materials were selected to provide a wide chemical range of test odors.

A crossover test design was used to compare the attractiveness of the different odors. Half of the locations contained Nasonov pheromone lures in the traps and the other locations contained one of the test odors in the traps. Traps were attached at heights of 2–4 m to the trunks and main branches of mesquite, or other suitable trees. On each survey of the trap sites, the lures were reversed (i.e., sites with Nasonov received test odor, and sites with test odor received Nasonov). The number of swarms attracted to traps of each type was recorded. Swarm attraction was determined by the presence of a swarm inside a nest cavity when opened. Occupied swarm traps were replaced with new traps. Treatments were compared statistically using a chi-squared test, with Yates' correction, one degree of freedom (Snedecor, 1956).

TABLE 1. ODORS AS HONEYBEE SWARM ATTRACTANTS

Test odor	Swarms in traps with			
	Nasonov	Test odor	Nas/Test	Prob [a]
None[b]	19	4	4.75	0.002
Linalool	21	7	3.0	0.02
Skatole	26	4	6.5	0.001
Clove oil	17	2	8.5	0.001
Wax moth pheromone	9	1	9.0	0.05

[a]Chi-square test using Yate's correction.
[b]Data from Schmidt (1994).

RESULTS

Traps containing Nasonov "pheromone" attracted almost five times as many swarms as control traps lacking any added odor (Table 1). All four test odors also attracted many fewer swarms than the Nasonov pheromone and were statistically less attractive. There were no statistical differences between any of the four test odors and the no-odor control. Thus, none of the test odors was more attractive to swarms than no odor at all, and none was repellent to swarms. The only odor that actually attracted swarms was the synthetic Nasonov blend.

DISCUSSION

The four chosen odors—linalool, skatole, clove oil, and wax moth pheromone— represent a broad cross section of odors from floral, plant, and animal sources and from a species associated in nature with bee colonies. These odors might have biological relevance and are olfactorily apparent to honeybees. The finding that none of the test odors significantly attracted more (or fewer) honeybee swarms to nest cavities than odorless cavities indicates that general odor cues play little role in the nest discovery and acceptance process. It is possible that with larger sample sizes a statistical difference between test odors (for example, between linalool and wax moth pheromone) might be observed, but that difference almost certainly will be small compared to the effect of the Nasonov blend. Moreover, the purpose of this investigation was not to distinguish subtle differences between individual odors but to determine if any of them had a similar attractiveness to honeybee swarms as Nasonov secretion. Clearly none of the tested odors elicited attractive responses resembling that of Nasonov.

The results indicate that general source or environmental odors appear unimportant to bees in the nest-seeking process. This suggests that scout bees

initially are capable of finding nest cavities without the aid of odor and that, except in extreme situations, odor is not likely to be a factor involved in the process. The odors selected for testing are familiar odors that are easily detected by bees; thus, lack of response is not a consequence of inability to detect the odor. These findings may relate to efforts to discover repellents for honeybee swarms. Because odors appear not to affect swarm decisions pertaining to attraction to or acceptance of nest cavities, most odors placed in locations where bees are not desired are unlikely to be effective.

Water- or syrup-seeking and nest-site-seeking behaviors of honeybees are fundamentally different and are governed by different rules and sensory information. Water- and syrup-seeking behaviors involve individual foragers, plus their recruits, and are associated with short-term acquisition of water and energy. Most water and nectar or syrup sources are odorous. The actual odor itself of these sources appears less important than the fact that an odor exists to help the foragers locate the liquid source (Wenner and Wells, 1990). If no odor exists, foraging honeybees will add an odor– Nasonov. Nasonov is added not as a pheromone (Wells et al., 1993), but simply as the only, or most available, odor to the bees. There likely is no effective pheromone used by bees to mark liquid sources.

Nest site seeking behavior, unlike foraging behavior, is associated with a long-term, almost permanent, decision that literally affects the survival of the entire colony. Unlike a poor foraging decision, which might mean that the honeybee colony suffers a small potential loss of food or water, the cost of a decision to select a poor nest site is extreme. In this situation, which demands a "collective decision" of many bees to ensure a proper decision, communication is essential. A pheromonal system is an ideal and effective means for such communication. Honeybees appear to meet this communication need via their Nasonov pheromone. Nasonov secretion meets all the criteria necessary to be a pheromone—it is released by individuals to attract other individuals of the species to a specific location, the receivers respond by being attracted to the pheromone source, and the pheromonal response apparently is not elicited by other known odors or secretions.

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